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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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1350 Connecticut Avenue NW
Washington, DC 20036

EXAMINER

PAK, YONG D

ART UNIT PAPER NUMBER

1652

DATE MAILED: 04/20/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary**Application No.**

10/031,146

Applicant(s)

HAUER ET AL.

Examiner

Yong D Pak

Art Unit

1652

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☐ Responsive to communication(s) filed on 11 February 2004.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-10, 12-14 and 16-24 is/are pending in the application.
- 4a) Of the above claim(s) 1-8, 13, 14, 16 and 19-24 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 9-10 and 12 is/are rejected.
- 7) ☒ Claim(s) 17 and 18 is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

The amendment filed on February 11, 2004, amending claims 9, 10, 12 and 17 and canceling claim 11, has been entered.

This application is a 371 of PCT/EP00/07253.

Claims 1-10, 12-14 and 16-24 are pending.

Election/Restrictions

Applicant's election with traverse of Group III in Paper No. 10 is acknowledged. The traversal is on the ground(s) that a special technical feature links Groups I-IV. Applicants argue that the examiner has not provided technical reasoning to support that at least one amino acid imparts modified substrate specificity. Munro et al. teach that "a single amino acid alteration may be sufficient to alter substrate specificity..." (page 409S).

The requirement is still deemed proper and is therefore made FINAL.

Claims 1-8, 13-14, 16 and 19-24 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in Paper No. 10.

Claim Objections

Claim 17, and its dependent claim, is objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim should refer to other claims in the

Art Unit: 1652

alternative only. The claim also references to two sets of claims having different features. See MPEP § 608.01(n). Accordingly, the claims have not been further treated on the merits.

Claims 17-18 are also objected to for being dependent on a non-elected claim.

Claim Rejections - 35 USC § 103

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 9-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wong et al. in view of Graham-Lorence et al.

Wong et al. teach a method of oxidizing N- or S-heterocyclic mono- or polynuclear aromatic compounds with a P450_{CAM}. Wong et al. also teach that replacement of any aromatic residues in the active site with a smaller, hydrophobic non-aromatic side-chain provides an "aromatic pocket" which could bind more hydrophobic substrates (page 3).

The difference between the reference of Wong et al. and the instant invention is that the reference of Wong et al. does not teach a method of oxidizing heterocyclic aromatic compounds using mutant P450_{BM-3}, cytochrome P450 monooxygenases from *Bacillus megaterium*.

Graham-Lorence et al. (form PTO-1449) teach a mutant P450_{BM-3} having a Val residue at position 87 of SEQ ID NO:2 (abstract). Phe-87 lies in the binding pocket and substitution of the residue with a non-aromatic hydrophobic residue Val provides an "aromatic pocket" which could bind more hydrophobic substrates, similar to the mutant of Wong et al. Also, since P450_{BM-3} is very well known and its structure and function are well characterized in the art, it is a good model in understanding substrate specificity of P450s (page 1127).

Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to use the mutant P450_{BM-3} of Graham-Lorence et al. to oxidize heterocyclic aromatic compounds. The motivation of using the mutant of Graham-Lorence et al. is out of convenience since the enzyme is well known and well characterized in the art. One of ordinary skill in the art would have had a reasonable expectation of success since the mutants of both Wong et al. and Graham-Lorence et al. have an "aromatic pocket".

Response to Arguments

Applicant's arguments February 11, 2004 with respect to the rejection of claims 9-10 and 17-18 under 35 U.S.C. 102(b) has been fully considered and are persuasive. The rejection of claims 9-10 and 17-18 under 35 U.S.C. 102(b) has been withdrawn due to amendment of claim 9.

Applicant's arguments filed on February 11, 2004 with respect to the rejection of claims 9-10 and 12 under 35 U.S.C. 112, 2nd paragraph and 35 U.S.C. 103 (a) have been fully considered but they are not persuasive.

Claim Rejections - 35 USC § 112

Claim 12 remains rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claim 12, clauses b) and c) are missing a conjunction. It is unclear if the mutations are in the alternative. For example, in clause c), it is unclear if the mutant contains Phe87Val, Leu188Gln and Ala74Gly or a combination of the three mutations.

Claims 9-10 and 12 remain rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: steps of converting non-heterocyclic aromatic substrates as claimed in claim 18 into heterocyclic aromatic compounds. The methods only recite steps of oxidizing heterocyclic aromatic compounds. Applicants argue that after steps a1) and a2) of claim 9 have been carried out, the non-heterocyclic aromatic substrates of claim 18 have been converted to heterocyclic aromatic compounds. The examiner disagrees. It is not clear how a linear alkane like n-hexane can be converted to a heterocyclic aromatic compound by simply incubating the oxidized n-hexane with a P450 monooxygenase.

Claim Rejections - 35 USC § 103

Claims 9-10 and 12 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Wong et al. in view of Graham-Lorence et al.

In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

Further, applicants argue that a skilled artisan would not have been motivated to substitute the teachings of Wong et al. with P450_{BM-3} since P450_{BM-3} and P450_{CAM} belong to different classes of P450 monooxygenase, having different substrate specificities and binding pocket. The examiner disagrees.

Even though the substrate specificity of wild type P450_{BM-} and P450_{BM-3} and binding pockets of P450_{CAM} and P450_{BM-3} are different, the teachings of Wong et al. can be applied to P450_{BM-3}. Like P450_{CAM}, P450_{BM-3} also has large aromatic amino acid in the active site. Graham-Lorence et al. teach that Phe-87 of P450_{BM-3} restricts substrate accessibility to the enzyme (page 1131) and also provides steric barrier to substrates (page 1133). Graham-Lorence et al. also teach that substrate selectivity depends on residues located in the active site (page 1135). Together with the teachings of Wong et

al., Graham-Lorence et al. and the knowledge available in P450_{BM-3} crystal structure, it would have been obvious to one of ordinary skill in the art that a substitution of a bulky amino acid in the binding pocket with an amino acid having a less hydrophobic side chain would lead to a more conducive binding pocket for hydrophobic substrates, like bulky aromatics, resulting in a mutant enzyme having a diverse range of substrate specificity. Therefore, it would have been obvious to one of ordinary skill in the art to oxidize other more bulkier substrates, like aromatics, with P450_{BM-3} mutants having a binding pocket more friendly to bulkier substrates.

Further, there are several reasons for using P450_{BM-3} instead of P450_{CAM}. P450_{BM-3} and P450_{CAM} are two of the most studied P450 monooxygenases. P450_{BM-3} is well characterized and its 3-D structure is widely available (Graham-Lorence et al. page 1127). The hemoprotein domain of P450_{BM-3} has the highest degree of functional and sequence homology to mammalian enzymes (Graham-Lorence et al. page 1127). Also, unlike P450_{CAM}, P450_{BM-3} is known in the art to be catalytically sufficient, requiring only NADPH and substrate for full activity since the reductase part of P450_{BM-3} is contained in the same polypeptide chain as the P450 (Munro et al. page 409S and Graham-Lorence et al. page 1127).

Therefore, one of ordinary skill in the art would have been motivated to devise a method of oxidizing various aromatic compounds with the mutants of Graham-Lorence et al.


No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yong Pak whose telephone number is 571-272-0935. The examiner can normally be reached 6:30 A.M. to 5:00 P.M. Monday through Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned are 703-872-9306 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Yong D. Pak
Patent Examiner



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